Original Article

Proximate composition and antimicrobial effect of *Ocimum gratissimum* on broiler gut microflora

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Abstract

The poultry industry is challenged by microbial infections, but the use of antibiotic growth promoters is restricted in many countries. This study is designed to assess the use of Ocimum gratissimum for the control of pathogenic gut microflora (*E. coli* and *Salmonella* sp). One hundred day old (ANAK 2000) commercial broilers were purchased and used for the experiment. The birds were brooded for seven days before random distribution to their respective treatments and replicates. Aqueous *Ocimum gratissimum* (0.11g/l) was administered to one set of 50 birds for 7 days (ocimum treatment), while the second sets of 50 birds which were not given the Ocimum extracts served as the control. Result show that the herb had a crude protein content of 4.56%, carbohydrate 13.40% and dry matter of 78.63%, but low in crude fibre (1.06%) and ash (1.21%). Prior to the administration of Ocimum extract the population of Salmonella was highest at the ileum (2.05 log cfu/g) followed by the crop (1.79 log cfu/g) and least at the caecum (1.77 log cfu/g). E. coli was highest at the ileum (2.05 log cfu/g), followed by the caecum (1.93 log cfu/g) and least at the crop (1.83 log cfu/g). Lactobacillus followed the pattern of *E. coli* but with different population, being highest at the ileum (1.95 log cfu/g) flowed by the caecum (1.90 log cfu/g) and least at the crop (1.79 log cfu/g). One week after the administration of Ocimum extracts to the chickens, the population of microbes in the gut decreased significantly ($P<0.05$) in relation to the control, suggesting the efficacy of Ocimum at reducing the population of enteric bacteria.

1. Introduction

Chicken meat is consumed by most people irrespective of race, tribe and religion. But the poultry enterprise is challenged by infection and high cost of feeds. Due to the problem of antibiotic resistance and possible carry over effect on consumers, the use of antibiotic growth promoter (AGP) is being restricted in many countries. Hence, alternative feedstock and drugs are increasing being sought after. Recently, there is increased research interest and awareness on the ethnoveterinary medicines, which involved livestock diseases generally[1-4] and poultry in particular[5,6]. For instance, the leaves of Christmas bush, *Alchornea cordifolia* have been used to control infections in human[7,8] and for the replacement of soya bean meal in the diet of New Zealand white rabbits[9]. Timibitei et al[10] demonstrated the use of *Alchornea cordifolia* in the modulation of gonads, serum enzymes, testosterone, estrogen and blood corpuscles of rabbits.

One of the herb/spice that is increasingly being used is alternative medicine in Asia, south America and Africa, particularly in India[11,12] and Nigeria[13,14]. *Ocimum gratissimum* Linn. Literature appears to be in conflict concerning the family of the plant. While some reported that *Ocimum gratissimum* belong to the Lamiaceae[13-18], other authors classified the plant as belonging to Labiata family [11,12,19-21]. The plant is variously called basil, clove basil, sweet basil, teabush, scent leaf and fever plant [13]. The plant has many medicinal uses including the treatment of upper headaches, neuralgia, abdominal pains, convulsion, tooth ache, regulation of menstruation, cure for prolapsed rectum, rheumatism and paralysis, seminal weakness, asthma, anaemia, pile, diabetes, bleeding, strokes[13-16,22]. It has been well documented that *O. gratissimum* exhibit antimicrobial properties against many infectious diseases including upper respiratory tract infections, cough and pneumonia, ear disease, conjunctivitis, tooth infection, skin disease (eczema, scabies, dermatitis), brounchitis, urogential infections including sexually transmitted diseases, and enteric diseases (diarrhea, typhoid fever and dysentery [13,19,21]. The plant has been reported to have broad antimicrobial properties against bacteria and fungi including *Escherichia coli, Pseudomaonas* species, *Klebsilla pneumonia, Shigella sonnei* and *Staphylococcus aureus* [12], *E. coli, Salmonella typhi, Shigella dysentariae* and *Staphylococcus aureus* [20], *E. coli, Pseudomonas aeroginosa, Salmonella typhi, Klebsiella pneumonia, proteus mirabilis* and *Candida albicans* [15], Listeria monocytogenes[23,24]. *O. gratissimum* is also effective against several species of fungi including *Trichophyton rubrum, T. mentagraphytes, Penicillium islandi* and *Candida albicans[24]*. The plant has been considered for the management of HIV/AIDS infections [12]. A detailed review of the medicinal, ethopharmacological, chemical and microbial properties of *O. gratissimum* reported by Prabhu et al[11].

The antimicrobial and other medicinal properties of *O. gratissimum* is linked to the presence of phytochemicals such as essential oils, flavonoids, steroids, phenols, alkaloids, tannins, mucilages, gums, resins, glucosides and glycosides, terpenes, sapronins and phytate [23,25]. Other authors linked the medicinal properties of *O. gratissimum* to the chemicals found in essential oil including eugenol, methyl eugenol, linalool, methyl cinnamate, Camphor, thymol, cis-and trans-ocimene, terpenes, germacrene, limsnene, camphene, citral, thujene and myrene[14-18,22,26]. To the best of our knowledge, the use of *O. gratissimum* in the control of poultry infectious has not been documented. Hence, this study is focused on the demonstration of the use of crude extract of *O.*

gratissimum for the control of pathogenic enteric bacteria (*Salmonella* and *E. coli*) and assesses their effects on beneficial lactobacillus species in the gastrointestinal tract (GIT) of broilers.

2. Materials and Methods

2.1 Source of Experimental Birds and preparation of brood house

One hundred day old (ANAK 2000) commercial broilers were purchased at CHI farm, Ibadan, Nigeria and transported to Niger Delta University Teaching and Research Farm where the experiment was carried out. Vittalyte was administered to the birds due to stress resulting from transportation. The brooder house and it environment was cleaned with detergent and disinfectant (Z-germicide) two weeks prior to the arrival of the birds. Electric bulb (200Watts) was used at the brooding stage as a source of heat and light. The feeders and drinkers were properly washed prior to brooding. The birds were brooded for seven days before random distribution to their respective treatments and replicates. The birds were fed with a commercial broiler starter diet for two weeks.

2.2 Source of *Ocimum gratissimum* and its preparation methods

Ocimum gratissimum used in this experiment were obtained from Swali market in Yenagoa Local Government Area of Bayelsa State, Nigeria. The *Ocimum gratissimum* was detached from the stalk and washed with clean water. *Ocimum gratissimum* leaves were allowed to drop excess water before shredding it. Five hundred grams (500g) was blended with an electric blender using 1.5 liters of distilled water. The aqueous *Ocimum gratissimum* solution was filtered with cheese cloth, after adding 3.0 liters of distilled water to the solution. The solution divided into 5 equal portions before IT was administered to the birds (Ocimum treatment). The procedure was repeated in 3 days interval. Another set of fifty birds were not given the aqueous *Ocimum gratissimum* solution (Control). The administration of aqueous *Ocimum gratissimum* was done on day seven.

2.3 Digesta Collection

Two birds per replicate were slaughtered prior to administration of aqueous *Ocimum gratissimum* and digesta from the gastrointestinal tracts i.e. - ileum, caecum and crop were collected into sterile container for microbial analysis. Digesta was collected again seven days after (day 14) administration of aqueous *Ocimum gratissimum* for a second set of microbial analysis.

2.4 Enumeration of microorganisms from the gastrointestinal tract of birds

The populations of microorganisms in the different samples were enumerated using serial dilution pour plate method of Pepper and Gerba [27](2005). About 1g of the sample was serially diluted in sterile distilled/deionized water and aliquots of the dilutions were ascetically plated into growth media; MRS Agar supplement with cycloheximide to enumerate total lactobacillus species. The medium were anaerobically incubated at 30ºC for 7 days. For the isolation of *E. coli* EMB Agar were employed and it was incubated aerobically at 30ºC for 24 hours. Salmonella-Shigella Agar was used to enumerate total *Salmonella* population. The medium was incubated aerobically at 30ºC for 24 hours, however, presence of black colonies indicates salmonella species. After incubation, the colonies that grew on the medium were counted and expressed as colony forming units (cfu)/g of the samples.

2.5 Proximate composition of *Ocimum gratissimum*

The proximate parameters determined for the plant including protein, lipid, ash, fiber, moisture etc. the guide provided by AOAC (1977) was used for the analysis[28].

2.6 Statistical analysis

Log transformations were carried out on total bacteria count (log CFU) before subjecting the results obtained to general linear model analysis using SPSS version 16. Mean separation was carried out using Duncan Multiple Range test.

3. Results and Discussion

The proximate composition of *O. gratissimum* used in the experiment is presented in Table 1. The herb had a crude protein content of 4.56%, carbohydrate 13.40% and dry matter of 78.63%, but low in crude fibre (1.06%) and ash (1.21%). Several authors have analyzed the composition of *O. gratissimum* and have obtained similar results. Belewu et al[29] in 2009 reported the composition of *O. gratissimum* to be 93.33% dry matter, 21.37% of moisture, 20.78% crude protein, 11.75% fat, 14.99% crude fiber and 3.58% ash. Mlitan et al[30] in 2014 reported the proximate composition of *O. gratissimum* as 9.10 – 9.80% protein 10.40 – 10.60% fat 10.80 – 11.16% moisture, 13.1 – 14.50% ash and 50.35 – 55.20% carbohydrate. Aluko et al[31] in 2012 reported the proximate composition of *O. canum* leaves to be 12% ash, 17% crude fibre and 7% crude fat. Emeka and Chimaobi [32] in 2012 reported 4.28 – 5.56% ash, 6.21% fat, 4.659.68 – 11.30% fibre, 30.35 – 34.05% moisture, 5.02 – 6.77% protein and 78.22 – 87.23% carbohydrate. Fagbohun et al[33] in 2012 reported the proximate composition of *O. gratissimum* leaves as follows; 5.11 – 7.77% ash, 5.04 – 6.54% moisture, 14.6 – 19.30% crude protein, 6.80 – 7.57% fat, 9.61 – 12.66% crude fibre and 50.08 – 56.16% carbohydrate. Adewole [34] in 2014 presented the proximate composition of *O. gratissimum* as follows; 10.30% moisture, 2.45% ash, 2.18% fat, 16.51% protein, 9.07% crude fibre and 58.89% carbohydrate. Efiong [35] in 2014 reported 4.43% protein, 2.7% fat, 4.2% crude fibre and 1.15% ash, which is comparable to our findings in this study. Idris et al [36] in 2007 reported 82% moisture, 3.33% protein, 8.50% lipid, 9.52% fibre and 64.98% carbohydrate with calorific value of 343.08 kcal/100g. Oboh et al [37] in 2009 reported the proximate composition of *O. gratissimum* as follows; 81.35% moisture, 1.2% protein and 0.57% ash. In addition, Abdurahman et al [38] in 2012 reported the presence of several minerals in the plant including 0.62mg/g calcium, 0.21mg/g fluoride, 1.6mg/g chromium, 14.83mg/g iron, o.47mg/g manganese and 2.14mg/g zinc. The differences in the proximate composition of *O. gratissimum* as reported by different authors may be due to the differences in the preparation and extraction of the plant components, which affected the moisture and ash contents.

Table 1 Proximate Composition of *Ocimum gratissimum*

Parameters	Proximate composition (g/100g)
Crude fibre	1.06
Dry matter content	78.63
Crude protein	4.56
Ash content	1.21
Moisture content	21.37
Carbohydrate	13.40
Percentage tannin	3.25

Prior to the administration of Ocimum extract the population diversity at the crop, ileum and caecum of the chicks were different (Table 2). The population of Salmonella was highest at the ileum (2.05 log cfu/g) followed by the crop (1.79 log cfu/g) and least at the caecum (1.77 log cfu/g). E. coli was highest at the ileum (2.05 log cfu/g), followed by the caecum (1.93 log cfu/g) and least at the crop (1.83 log cfu/g). *Lactobacillus* followed the pattern of *E. coli* but with different population, being highest at the ileum (1.95 log cfu/g) flowed by the caecum (1.90 log cfu/g) and least at the crop (1.79 log cfu/g). In a previous study Ohimain and Ofongo[39] in 2013 reported different populations of microbes in the crop, ileum and caecum of broilers. The population of *E.coli* was 7.05 log cfu/g at the crop, 7.02 log cfu/g at the ileum and 6.98 log cfu/g at the caecum. Lactobacillus has 6.63 log cfu/g at the crop, 6.66 log cfu/g at the ileum and 6.73 log cfu/g at the caecum.

Table 2: Microbial population from the gastrointestinal tracts of the birds before administration of aqueous *Ocimum gratissimum*

Bacterial Species	*Salmonella*	*Escherichia coli*	*Lactobacillus*
CROP	1.79b	1.83a	1. 79a
ILEUM	2.05c	2.05c	1.95c
CAECUM	1.77a	1.93b	1.90b

Along the columns, means with different alphabets are significantly different according to the Duncan statistics ($p<0.05$)

Generally, the population of microbes in the GIT increases with age until a stable population is reached. One week after the administration of Ocimum extracts to the chickens, the population of microbes in the GIT decreased significantly (P<0.05) in relation to the control (Table 3), suggesting the efficacy of Ocimum at reducing the population of enteric bacteria. Lower population of *Salmonella*, *E. coli* and *Lactobacillus* was recorded at the crop, ileum and caecum (P<0.05). The result therefore shows that the antibiotic property of *O. gratissimum* does not discriminate between pathogenic (*Salmonella* and *E.coli)* and beneficial microbes (*Lactobacillus*) hence its use has to be applied with caution. Many authors have reported the efficacy of Ocimum against *Salmonella* [22,40-42], *lactobacillus* [43,44] and *E. coli* infections[45-51].

Table 3: Microbial population in the gastrointestinal tracts of broilers after administration of aqueous *Ocimum gratissimum* extracts

Bacterial Species	Ocimum Treatment, Log cfu/g	Control, Log cfu/g	SEM	*P.VALUE*
CROP				
Salmonella	1.85[b]	2.20[a]	0.106	0.016**
Escherichia coli	1.93[b]	2.06 a	0.156	0.050*
Lactobacillus	1.95[b]	2.14 a	0.075	0.030**
ILEUM				
Salmonella	2.04[b]	2.27[a]	0.113	0.041**
Escherichia coli	1.93[b]	2.09[a]	0.120	0.000***
Lactobacillus	1.88[b]	2.08[a]	0.096	0.010***
CEACUM				
Salmonella	1.82[b]	1.95[a]	0.139	0.030**
Escherichia coli	1.97[b]	2.17[a]	0.122	0.000***
Lactobacillus	1.67[b]	1.95[a]	0.125	0.020***

Along the rows, means with different alphabets are significantly different according to the Duncan statistics (p<0.05)

4. Conclusion

This study tested the effects of *Ocimum gratissimum* on the gut microflora of broiler chickens. The administration of aqueous extracts of *Ocimum gratissimum* WAS found to be able to control the population of E. coli and salmonella, but also negatively affected beneficial microbes like lactobacillus. Hence, we conclude by recommending the use of the herb for the control of microbial infections caused by detrimental gut microbes, while enhancing the probiotic effects of lactobacillus.

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